

## SEQUENCE LISTING

<110> Korea Research Institute of Bioscience and Biotechnology

<120> Method for screening of a lipase having improved enzymatic activity using yeast surface display vector and the lipase

<130> 3fpo-07-05

<150> KR 2002-55575

<151> 2002-09-13

<160> 18

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<210> 1

<211> 27

<212> DNA

<213> Artificial Sequence

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<223> CALB primer 1

<400> 1

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27

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<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 2

<400> 2

gcggatcctc aggggtgac gat

23

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<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 3

<400> 3

gcggatccgg gggtgacgat gccggag

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<210> 4

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> GPD-err primer

<400> 4

gcagagctaa ccaataagg

19

<210> 5

<211> 19

<212> DNA

<213> Artificial Sequence

PCT/KR03/01820  
RO/KR 04.11.2003

<220>  
<223> T-0 primer

<400> 5

tgcagttgaa cacaaccac

19

<210> 6

<211> 1023

<212> DNA

<213> Candida antarctica

<220>

<221> sig\_peptide

<222> (-51)..(-1)

<223> secretion signal

<400> 6

atgaatatat ttacatatt ttgttttg ctgtcattcg ttcaaggtac cgccactcccc

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tttgtgaagc gtctgcctc cggttcggac cctgccttt cgcatccccaa gtcggtgctc

69

qatqcqqqtc tqacctqccca qqqtqcttcq ccatcctcq tctccaaacc catccttc

129

gtccccggaa ccggcaccac aggtccacag tcgttcgact cqaactqqat ccccctctt

189

gcgccagctgg gttacacacc ctgctggatc tcaccccccgc cgttcatqct caacqacacc

249

caaggtaaca cqqagtacat qqtcaacqcc atcaccacqc tctacqctqq ttcccccaac

309

aacaadcttc ccgtqctcac cttagtccccaaq qqtatgtcttgg ttgcacatgtq qgggtctqacc

369

ttcttccccca qtatcaaggc caaqgtcqat cqacttatqq cctttacqccc cqactacaac

429

qqcaccqtc tgcqccqccc tctcqatqca ctcqcqgta qtqccaccctc cqtagqqcad

489

caaaccaccc gttcggca cactaccgca ctccgaaacg caggtggct gacccagatc 549  
gtgcccacca ccaacctcta ctggcgacc gacgagatcg tttaggcctca ggtgtccaac 609  
tcgcccactcg actcatccta cctcttcaac gggagaacg tccaggcaca ggctgtgtgt 669  
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gactgcaacc ctctccgc caatgatctg actcccgagc aaaaggtcgc cgccgctgac 849  
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ccc 972

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<213> Candida antarctica

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<222> (-51)..(-1)  
<223> secretion signal

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gatgcgggtc tgacctgcca aggtgctcg ccatcctcg tctccaaacc catcctctc 129

gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat ccccctctct	189
gcgcagctgg gttacacacc ctgctggatc tcaccccgcc cgltcatgct caacgacacc	249
caggtcaaca cgaggatcacat ggtcaacgccc atcaccacgc tctacgctgg ttccggcaac	309
aacaagcttc ccgtgctcac ctggtcccag ggtggctgg ttgcacagtg gggctgacc	369
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gtgcccacca ccaacctcta ctcggcgacc gacgagatcg tttagcctca ggtgtccaa	609
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gactgcaacc ctctccgc caatgatctg actcccgagc aaaaggctgc cgccgctcg	849
ctccggcgc cggcggctgc agccatcgatg gcgggtccaa agcagaactg cgagcccgac	909
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ccc	972

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 <213> Candida antarctica

<220>  
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<222> (-51)..(-1)  
<223> secretion signal

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ttggtaaagc gtctgccttc cggttggac cctgccttt cgcagccaa gtcgggctc 69

gatgcgggtc tgacctgcca gggtgctcg ccacccctcg tctccaaacc catcctctc 129

gtccccggaa cggcaccac aggtccacag tcgttcact cgaactggat ccccctct 189

gcccggctgg gttcacacacc ctgctggatc tcaccccgcc cggtcatgtt caacgacacc 249

caggtaaca cggagtacat ggtcaacgcc atcaccacgc tctacgtgg ttccggcaac 309

aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtgg gggctgacc 369

ttctccca gtagcaggta caaggtcgat cgacttatgg ctttgcgcc cgactacaag 429

ggcacccgtcc tcggccccc tctcgatgca ctgcggta gtgcaccctc cgtatggcag 489

caaaccaccg gttcgact cactaccgca ctccgaaacg cagggtgtt gacccagatc 549

gtgcccacca ccaacctcta ctggcgacc gacgagatcg tttagccca ggtgtccaa 609

tgcactcg actcatccta cctttcaac gaaaagaacg tccaggcaca ggctgtgt 669

ggccgcagt tcgtcatgca ccatgcaggc tcgctcacct cgcaatctc ctacgtcg 729

ggtcgatccg ccctgcgctc caccacggc caggctcgta gtgcagacta tggcattacg 789

gactgcaacc ctctccgc caatgatctg actcccgagc aaaaggctgc cgccgctcg 849

ctcctggcgcc cggcggctgc agccatcgta gcgggtccaa agcagaactg cgagccgac 909

ctcatgccct acgccccccc cttgcagta ggcaaaagga cctgctccgg catcgtcacc 969

ccc 972

<210> 9

<211> 341

<212> PRT

<213> Candida antarctica

<220>

<221> SIGNAL

<222> (-24)..(-8)

<223> secretion signal

<400> 9

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly

-24 -20 -15 -10

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala

-5 1 6

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly

11 16 21

Ala Ser Pro Ser Ser Val Lys Pro Ile Leu Leu Val Pro Gly Thr

26 31 36

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser

41 46 51 56

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met

61 66 71

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr

76 81 86

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp

91 96 101

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser

106 111 116

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys

121 126 131 136

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro

141 146 151

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg

156 161 166

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser

171 176 181

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp

186 191 196

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys

201 206 211 216

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe

221 226 231

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala

236 241 246

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn

251 256 261

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Leu Pro Ala Pro

266

271

276

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp

281

286

291

296

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser

301

306

311

Gly Ile Val Thr Pro

316

<210> 10

<211> 341

<212> PRT

<213> Candida antarctica

<220>

<221> SIGNAL

<222> (-24)..(-8)

<223> secretion signal

<400> 10

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly

-24

-20

-15

-10

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala

-5

1

6

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly

11

16

21

Ala Ser Pro Ser Ser Val Lys Pro Ile Leu Leu Val Pro Gly Thr

26

31

36

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser

41 46 51 56

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met

61 66 71

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr

76 81 86

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp

91 96 101

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser

106 111 116

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys

121 126 131 136

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro

141 146 151

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg

156 161 166

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser

171 176 181

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp

186 191 196

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys

201 206 211 216

Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe

221 226 231

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala

236 241 246

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn

251 256 261

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro

266 271 276

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp

281 286 291 296

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser

301 306 311

Gly Ile Val Thr Pro

316

<210> 11

<211> 341

<212> PRT

<213> Candida antarctica

<220>

<221> SIGNAL

<222> (-24)..(-1)

<223> secretion signal

<400> 11

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly

-24 -20 -15 -10

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala

-5 1 6

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly

11 16 21

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr

26 31 36

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser

41 46 51 56

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met

61 66 71

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr

76 81 86

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp

91 96 101

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser

106 111 116

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys

121 126 131 136

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro

141 146 151

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg

156 161 166

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser

171 176 181

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp

186 191 196

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys

201 206 211 216

Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe

221

226

231

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala

236

241

246

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn

251

256

261

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Leu Ala Pro

266

271

276

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp

281

286

291

296

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser

301

306

311

Gly Ile Val Thr Pro

316

<210> 12

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 4

<400> 12

ctcataatgct accttccgggt tcggac

26

<210> 13

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> α-amylase secretion signal

<400> 13

Met Met Val Ala Trp Trp Ser Leu Phe Leu Tyr Gly Leu Gln Val Ala

1 5 10 15

Ala Pro Ala Leu Ala

20

<210> 14

<211> 317

<212> PRT

<213> Candida antarctica

<400> 14

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Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys

20 25 30

Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe

35 40 45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys

50 55 60

Trp Ile Ser Pro Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr

65 70 75 80

Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn

85

90

95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln

100

105

110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu

115

120

125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu

130

135

140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly

145

150

155

160

Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile

165

170

175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro

180

185

190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys

195

200

205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Leu Phe Val Ile Asp His

210

215

220

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala

225

230

235

240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr

245

250

255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val

260

265

270

Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ala Ile Val Ala Gly

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Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe

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Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro

305 310 315

<210> 15

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> LQ53 primer

<400> 15

gctgtgttg ggccgcagtt cgtcatcg 28

<210> 16

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> LQ35 primer

<400> 16

gcatggtcga tgacgaactg cggcccacac 30

<210> 17

<211> 30

<212> DNA

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<213> Artificial Sequence

<220>

<223> LP53 primer

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gtcgccgccc ctgcgtcccc ggccgcggcg

30

<210> 18

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> LP35 primer

<400> 18

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29